B1 Cont'd

34. The method of claim 14, performed in multi well plates of 384 or 96 wells.

35. The aqueous buffer of claim 25, wherein the C_1 - C_4 alcohols are ethanol or isopropanol, and the buffer substance is a ω-amino acid.

36. The kit of claim 26, wherein the auxiliary materials are columns with or without silicaceous material, suspension of silicaceous material, additional ~~buffers, or instruction manual.~~

B2 Sub C1

37 ~~36.~~ The kit of claim 36, wherein the additional buffers are resuspension buffers, lysis buffers, washing buffers, or elution buffers.

REMARKS

New claims 14-36 are presented, hereby.

Support for claims 14-36 is found in the original claims and additionally, with respect to present claim 23, in the paragraph bridging pages 16-17 of the specification. Thus, claims 14-36 contain the subject matter of original claims 1-13, revised to more clearly define the invention.

Claims were rejected under 35 USC 112, ¶1, for alleged lack of enablement. Reconsideration is requested.

According to the statement of rejection, the claims are overly broad with respect to performing the claimed method at "pH> 8 . . . in the presence of at least one chaotropic substance." To overcome the rejection, the statement of rejection would apparently require (a) inserting an upper limit to the pH value and (b) narrowing "chaotropic agent" to embodiments described in the specification. The rejection cannot be maintained as it does not apply the correct standards for determining enablement under §112, ¶1, of the statute.

The claims need not be limited to only those embodiments of "chaotropic agent" disclosed in the specification. Lack of enablement is not demonstrated merely because the claim scope might, theoretically, cover embodiments that do not work; the function of the claims is not to specifically exclude possibly inoperative embodiments. *Atlas Powder v. E.I. du Pont de Nemours Co.*, 224 USPQ 409 (Fed. Cir. 1984). Lack of enablement under §112 is not established by mere allegations of undue breadth, that is, by merely arguing that claims read on non-disclosed embodiments. *Horton v. Stevens*, 7 USPQ2d 1245 (BPA & I 1988).

In order to satisfy the requirements of §112, first paragraph, "it is not necessary to embrace in the claims or describe in the specification all possible forms in which the claimed principle may be reduced to practice." *Smith v. Snow*, 294 U.S. 1, 11 (1935). The law does not require an applicant to describe in his specification every conceivable embodiment of the invention. *SRI Int'l v. Matsushita Elec. Corp. of America*, 227 USPQ 577, 586 (Fed. Cir. 1985). Moreover, while working examples drawn to specific embodiments may be desirable, they are not *required* in order to satisfy enablement under §112. *In re Strahilevitz*, 212 USPQ 561 (CCPA 1982).

The statement of rejection relies on concerns that not all chaotropic agents would work in the presently claimed invention. That not all chaotropic agents would work fails to establish lack of enablement under §112, ¶1. The "use of materials which might prevent achievement of the [claim] objective . . . can hardly be said to be within the scope of the claims." *In re Geerdes*, 180 USPQ 789, 793 (CCPA 1974). Section 112 enablement is satisfied when generic claims cover thousands of end products, some of which may not be operative (i.e., may not work for the stated

purpose of the claimed invention). *Atlas Powder v. E. I. duPont de Nemours*, 224 USPQ 409 (Fed. Cir. 1984). The mere presence of some non-working embodiments within a generic claim does not justify a rejection for lack of enablement under §112, first paragraph.

> As we have said before, it is almost always possible to so construe a claim as to have it read on non-working embodiments, In re Cook, 58 CCPA 1049, 1054, 439 F.2d 730, 734, 169 USPQ 298, 301 (1971), but the alternative of requiring an applicant to be so specific in his claims "as to exclude materials known to be inoperative . . . would result in claims which would fail to comply with 35 U.S.C 112, second paragraph, because they would be so detailed as to obscure, rather than to particularly point out and distinctly claim, the invention. In re Meyers, 56 CCPA 1129, 410 F.2d 420, 161 USPQ 668 (1969), quoted with approval in In re Anderson, 471 F.2d 1237, 176 USPQ 331 (CCPA 1973).

In re Smythe, 178 USPQ 279, 286 (CCPA 1973)(*emphasis in original*).

With respect to the pH, the upper limit need not be recited in the claims to satisfy the requirements of enablement. The issue presented concerns how to practice the invention, which is the function of the specification, not the claims. *In re Roberts*, 176 USPQ 313, 315 (CCPA 1973).

Reconsideration is requested wityh respect to the rejection under 35 USC 112, ¶2, for alleged indefiniteness of the claims, in view of the claim changes effected, hereby.

Claims 1-6 and 8-11 were rejected under 35 USC 103(a) based on the combined teachings of Little and Marko. Claims 7 and 8 were rejected under 35 USC 103(a) based on the combined teachings of Little, Marko, Smith and Segel (the rejection names "claims 1-11" as being rejected, but only claims 7 and 8 are discussed in the body of the rejection). Claims 12 and 13 were rejected under 35 USC 103(a) based on the combined teachings of Bastian (either the U.S. patent or the

corresponding published International application) and Segel. Reconsideration is requested with respect to the aforesaid rejections.

The presently claimed invention is concerned with a method for separating circular nucleic acids from a mixture having different speciess of nucleic acids. Many techniques in biology/biochemistry do require pure nucleic acids in general and Plasmid DNA in particular.

Plasmids are a preferred tool in molecular biology due to the fact that they replicate autonomously from the chromosomal DNA and that they - in other than chromosomal DNA - can be isolated from bacteria cells in intact form. They can be hdrolysed at specific recognition sequences with commercially available restriction endonucleases, joined with fragment of foreign DNA, amplified with DNA polymerases and transferred into suitable cells.

The isolation of plasmid DNA is therefore a prerequisite for subsequent molecular biological experiments, such as PCR reactions, sequencing reaction, cloning reactions, restriction hydrolyses, transformations and transfections.

Several methods for the isolation of plasmid DNA from bacterial cells are known. Common to all these methods is that they follow the scheme:

1. Formation of cleared lysate and
2. Purification of plasmid DNA from the cleared lysate.

The steps for the formation of cleared lysate are almost identical among the different methods. Characcteristic differences occur during the purification of plasmid DNA from the cleared lysate.

The formation of cleared lysate comprises:

- cell lysis
- precipitation of cellular components and, subsequently,
- removal of the precipitate from the plasmid containing solution to form cleared lysate.

Cell lysis is usually realized under alkaline conditions in the presence of sodium dodecylsulfate resulting in bacterial crude lysate. To precipitate cellular components such as chromosomal DNA, proteins, cellular debris etc., a potassium or sodium acetate buffer is added to the crude lysate, which adjusts the mixture to slightly acidic pH (4.8 to 5.0). Plasmid DNA does not precipitate under these conditions, so it remains in the supematant. In order to form a cleared lysate, the precipitate is removed from the plasmid containing solution, either by centrifugation (Sambrook J., Fritsch E.F. and Maniatis T. (1989), "Molecular Cloning. A Laboratory Manual", pp. 7.49 to 7.50, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY Maniatis, Laboratory Manual), filtration (EP 0 616 638 B1), or magnetic separation using magnetically attractable beads, which do not specifically bind the precipitate (US Patent 5,681,946 and US Patent 5,523,231).

All methods for the isolation of plasmid DNA from bacteria known from the sate of the art at the date of invention comprise the formation of cleared lysate. All known methods for clearing the bacterial lysate after precipitation of cellular particles, e.g., centrifugation, filtration, and magnetic separation using magnetically attractable beads, which do not specifically bind the precipitate, display considerable disadvantages. Lysate clearing by centrifugation is usually the

most time consuming step in the corresponding plasmid isolation protocols. Lysate clearing by filtration, however, requires the application of an appropriate filter, which is commonly a major cost factor of the corresponding protocol.

The presently claimed invention provides a process for the isolation of circular nucleic acids, in particular plasmid DNA, directly from sources containing such nucleic acids. In particular, the nucleic acids are isolated from bacterial crude lysate, avoiding the need to form a cleared lysate. The presently claimed invention provides a method of separating circular nucleic acids from a mixture containing different species of nucleic acids other than circular nucleic acids, which avoids the drawbacks of the prior art.

According to the presently claimed invention, the mixture containing circular nucleic acids is treated under alkaline conditions at a pH > 8 with a solid matrix consisting essentially of a silica material in the presence of at least one chaotropic substance. In particular, the alkaline pH can be at least 9 or about 10. The upper pH value is limited by the individual circular nucleic acid. It depends on various conditions, for example, on the amount and the kind of chaotropic salt present. It is within the normal skill of the artisan how to optimize the respective separation conditions given the teachings of the presently claimed invention.

Preferably, the circular nucleic acid is double stranded DNA, particularly a plasmid.

<u>The cited state of the art:</u>

The statement of rejection alleges that each of Little and Marko would fully meet the presently claimed invention. Marko et al. disclose the use of a chaotropic substance and powder

in a procedure for the isolation or pre purified plasmid nucleic acid. The nucleic acid is already purified prior to being contacted with the chaotrope and the siliceous solid phase. Based on these teachings, the skilled person would only be motivated to incorporate pre-treatment steps to purify the nucleic acid prior to contacting it with the chaotrope and a siliceous solid phase to get, firstly, a clear lysate.

In particular, Marco et al., disclose a process for the purification of plasmid-DNA, which in the first step needs essentially the formation of a cleared lysate. After resuspension of the bacterial cells in a buffer (PEBI), lysis, and denaturation of chromosomal DNA by addition of buffer (PEB II) certifugation (precipitation of cell debris and chromosomal DNA,) a cleared lysate is formed. The result of this step is that the chromosomal - i.e, non circular DNA - is part of the precipitate; whereas, the plasmid DNA (circular DNA) is part of the supematant.

The step relevant to the separation of the chromosomal DNA is represented by the addition of buffer PEB III (working at a pH of approx. 6).

The further purification of the plasmid DNA starts from this cleared lysate.

In contrast to Marko, the presently claimed invention provides a method of plasmid purification from bacterial crude lysate comprising the use of novel buffer compositions which selectively bind plasmid DNA to silica material, but not chromosomal DNA or other cellular impurities present in the crude lysate. The selective binding of plasmid DNA to silica material in the presence of linear chromosomal DNA fragments and other cellular impurities is achieved by

adjusting alkaline binding conditions in the presence of high concentrations of chaotropic substances.

Thus, Marko does not in any way suggest the presently claimed invention. Accordingly, the process at the presently claimed invention is clearly patentable over Marko. Secondary references do not cure the fatal deficiencies of Marko.

Regarding US Patent 5,075,430 (Little), it is firstly to be noted that this invention starts with pre-purified DNA. Little is concerned with a process for the purification of single-stranded plasmid and other DNA, by immobilizing the DNA onto diatomaceous earth in the presence of a chaotropic agent and eluting the DNA with water containing a low salt buffer. The resulting purified DNA is biologically active. Also, included in the Little invention is a process for the immobilization of DNA onto diatomaceous earth in the presence of a chaotropic agent.

From the examples as well as from the description [Little column 5, lines 43-64] it clearly can be seen that Little needs pre-treated DNA.

> Plasmid DNA from mini-prep lysates can be purified using the process of the presently claimed invention. Lysis methods which work with this process include Triton_Lysozyme Lysis (Ausubel et al., ads. Current Protocols in Molecular Biology, John Wiley & Sons, 1987 pp. 1.7.5-1.7.7), Triton Lysis-Boiling (Holmes et al., 1981. Analyt. Biochem. 114:193-197) and the cleared lysate method employing SDS/NaOH (Maniatis et al., eds., Molecular Cloning, Cold Spring Harbor Laboratory. 1982. P. 90), the disclosures of each of which are incorporated herein by reference. The amount of RNA contamination occurring will depend on the number of chaotrope and ethanol washes used. If using the cleared lysis method, the process of the invention will begin with a nucleic acid pellet (usually the first isopropanol pellet is adequate). If using the boiling method or the lysozyme method, the process may begin with a resuspended nucleic acid pellet or with the supernatants derived from the centrifugation

> step used to eliminate the cellular debris. If dried nucleic acid pellets are used, they are resuspended well in TE or water, and any clumps of undissolved protein are centrifuged out.

As a further alternative DNA from agarose gel slices can be used in the process of Little. This leads back to Marko, also using DNA from an agarose gel.

Moreover, as a matter of completeness only, it is submitted that isolation of DNA from agarose is not as complicated as the isolation of nucleic acid from a complex biological starting material. (The nucleic acid present in agarose has already been isolated from the biological material in which it was originally present.) In agarose the DNA is already pure and free of other cell constituents that might possibly harm the nucleic acid. Furthermmore, the DNA present in gels is usually of a defined length (e.g. a restriction fragment) and not as complex and vulnerable as the nucleic acid present in the biological material other cell components are also present. Especially when cells are lysed these other components might damage the nucleic acid. Someone skilled in the art would, for example, expect the nucleases present in such samples, especially when the cells are lysed, to damage the nucleic acid. The nucleic acid would be cleaved or broken down and thus unsuitable for further use. This is a serious problem with nucleic acid extraction from biological material.

Based on Little a skilled person would, therefore, expect serious problems when the Little method would be applied to non pre-treated biological material. However, apparently, with the method according to the presently claimed invention, the nuclease activity is significantly reduced or the DNA can be isolated from its environment before being broken down by the nucleases.

Claim 8 was rejected as being obvious in further reliance of Segel [Biochemical calculations]. The statement of rejection argued that it would have been obvious to add the aqueous solution of an amphoteric substance in place of the buffer used in each of the Little or Marko citation to obtain a pH of 8 to 12 in the resulting mixture.

As was shown above, neither Little nor Marko render the presently claimed invention obvious. Accordingly, it cannot be seen that a combination of the teaching of Marko or Little with the mere name of an amphoteric compound together with its pKa value, described in Segel, would lead to the presently claimed invention.

Claims 12 and 13 were unpatentable over Bastian et al. [WO 95/21849] in view of Segel. The statement of rejection maintained that the thiocyanate reagents disclosed in Bastian "must be considered" per se the "sodium thiocyanate" component of the "aqueous buffer" in the Kit-of-parts-claims 12 and 13.

However, it cannot be seen that this line of argumentation is open for the statement of rejection. The correct approach with regard to these two claims is: whether the skilled artisan would have replaced the thiocyanate compounds disclosed in the Bastian citation to achieve the aim of the presently claimed invention with a reasonable expectation of success. The answer with regard to the present application must clearly be no.

Applicants submit that the statement of rejection relies on an "ex-post-facto" analysis, which is not allowed in making an obviousness determination under §103. *In re Deminski* 230 USPQ 313 (CCPA 1986).

Favorable action is requested.

Respectfully submitted,

JACOBSON HOLMAN PLLC

By William E. Player
Registration No. 31,409

400 Seventh Street, N.W.
Washington, D.C. 20004
Telephone: (202) 638-6666
Atty. Docket No.: P63712US0
Date: August 14, 2001
WEP:vjb
R:\HOME\VBYERS\2001\August\P63712amd.wpd-